Complete Donor T Cell Chimerism Predicts Lower Relapse Incidence after Standard Double Umbilical Cord Blood Reduced-Intensity Conditioning Regimen Allogeneic Transplantation in Adults

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Double umbilical cord blood (dUCB) allogeneic transplantation after a low-dose total body irradiation, cyclophosphamide, and fludarabine (TCF)—based reduced-intensity conditioning regimen (RIC) is increasingly used in adults lacking a suitable related or unrelated donor. Currently, there are little data regarding the long-term outcome of $CD3^+$ T cell chimerism (TCC) in this particular setting. Thirty-six adults with various hematological diseases who received dUCB allogeneic transplants conditioned with TCF were included in this retrospective study. Peripheral blood $CD3^+$ TCC was considered until day +100 after transplantation to determine the impact of full versus mixed chimerism on long-term outcomes. Twenty-nine and 7 patients were documented with full and mixed $CD3^+$ TCC, respectively, within the first 100 days after transplantation. With a median follow-up of 36 months, 3 year-overall survival (OS), disease-free survival (DFS), and cumulative incidence of relapse (CIR) were 61%, (95% confidence interval [CI], 43% to 75%); 50% (95% CI, 32.5% to 66%), and 28% (95% CI, 16% to 44%), respectively. In univariate analysis, a full $CD3^+$ TCC was associated with a better 3-year DFS: 59% (95% CI, 39% to 75.5%) versus 14% (95% CI, 7% to 46%); hazard ratio (HR), .24 (.09 to .65); $P = .005$ and a lower CIR: 24% (95% CI, 21.5% to 57%) versus 78% (95% CI, 52% to 99%); HR, .18 (.05 to .50); $P = .004$. In multivariate analysis, a full $CD3^+$ TCC remained associated with a lower CIR (HR, .17 [.028 to .99]; $P = .049$). $CD3^+$ TCC has no impact on graft-versus-host disease and nonrelapse mortality in this study. In conclusion, here, full $CD3^+$ TCC was independently associated with a lower risk of relapse in adults receiving a dUCB TCF RIC allogeneic transplantation. This highlights the need to develop immunotherapy approaches allowing for early conversion to full chimerism after this type of transplantation.

INTRODUCTION

The goal of allogeneic transplantation for malignant hematological diseases is complete eradication of tumor cells together with the development of complete donor chimerism. Chimerism is a measure of the number of donor and recipient cells in the host after infusion of the allogeneic stem cells. Among different techniques of measurement, polymerase chain reaction of short tandem repeats using fluorescent amplification is currently considered the most sensitive method to detect donor cells [1]. Myeloablative allotransplantations are generally associated with full donor chimerism, whereas mixed chimerism occurs frequently after reduced-intensity conditioning (RIC) regimens or T cell—depleted allografts. At present, the significance of mixed chimerism is not clear, as residual host cells may represent normal hematopoiesis without deleterious impact on outcome. On the other hand, it may be also predictive of secondary graft failure [2,3] or disease relapse due to the persistence of host tumor cells together with a lower risk of acute [4-6] or chronic graft-versus-host disease (GVHD) [7,8]. Thus, many investigators consider that patients with mixed chimerism are potential candidates for adoptive immunotherapy. Another point to consider is whether the chimerism is detected in whole blood or bone marrow and if it is lineage specific. Currently, it is recommended to use peripheral blood cells and lineage-specific chimerism, especially $CD3^+$ T cell chimerism (TCC), as a more sensitive assay, especially in the nonmyeloablative setting [9].

Double umbilical cord blood (dUCB) transplantation after RIC represents a valid option for curing adult patients with hematological disorders without related or unrelated donors [10-12]. The so-called TCF RIC regimen developed by the Minneapolis group is 1 of the most widely used regimens for these patients [13]. It combines low-dose total body irradiation (2 Grays), cyclophosphamide 50 mg/kg for 1 day, and fludarabine 40 mg/m^2 for 5 days (200 mg/m^2 total dose) and provides similar results compared with peripheral blood stem cells RIC allogeneic transplantation [14]. Among factors that could influence outcomes after dUCB allotransplant in

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Table 1
Patients, Sustained Cord Blood, and Transplantation Characteristics

Characteristic	Full TCC (n = 29)		Mixed TCC (n = 7)		*P* Value
	No. of Patients	%	No. of Patients	%	
Patients characteristics					
Age at transplantation, median (range), yr	57 (22-69)		47 (17-64)		NS
Sex: female	14	48	3	43	NS
Hematological malignancy: Lymphoid/myeloid	14/15	48/52	3/4	43/57	NS
Status at transplantation: CR/PR	23/6	79/21	6/1	86/14	NS
Time to transplantation, median (range), d	395 (137-5645)		216 (92-604)		NS
Cord blood characteristics					
Age of cord blood, median (range), mo	31 (9-165)		116 (23-140)		NS
Matching cordon with patient					NS
4/6	10	35	3	43	
5/6	19	65	3	43	
6/6	0	0	1	14	
Number of TNC before and after thawing, respectively, median (range), $\times 10^8$/kg	.28 (.16-.455); .248 (.157-.406)		.222 (.135-.492); .22 (.11-.392)		NS
Number of $CD34^+$ cell before and after thawing, respectively, median (range), $\times 10^6$/kg	.066 (.022-.215); .043 (.02-.2)		.078 (.031-.427); .041 (.019-.259)		NS
Mismatch between cord blood and patient					
Sex	14	48	3	43	NS
Serology CMV	13	45	3	43	NS
ABO	16	55	2	28	NS
Rhesus	22	76	6	86	NS
Graft					
Neutrophil count recovery >.5 g/L, median (range), d	17 (6-32)		11 (7-20)		NS
Platelet recovery > 20 g/L, median (range), d	41 (0-164)		31 (0-67)		NS
Acute GVHD (grade II-IV/grade III-IV)	19 (12/6)	65 (41/21)	4 (3/1)	57 (43/14)	NS
Chronic GVHD (Limited/extensive)	11 (8/3)	38 (28/10)	3 (2/1)	43 (28/14)	NS
Chimerism					
Rate, median (range), %	100 (96-100)		82 (14-94)		<.001

NS indicates not significant; CR, complete remission; PR, partial remission; CMV, cytomegalovirus.

adults, chimerism has been poorly investigated, thus far. Indeed, if a favorable impact of full donor chimerism on hematopoietic recovery or pre-engraftment syndrome has been already reported, no data regarding influence of mixed versus full TCC chimerism on long-term outcomes are yet available after dUCB RIC transplantations in adults [15,16].

This study aimed to investigate the influence of full versus mixed TCC on various outcomes in a cohort of adult patients who engrafted after a standard dUCB TCF allotransplantation.

PATIENTS AND METHODS

Study Design, Patients' Characteristics, and Graft Selection Criteria

All adult patients (17 to 70 years old) in our institution who engrafted after a dUCB TCF allotransplantation and for whom at least 1 TCC was assessed within the first 100 days after transplantation were eligible for this study. TCF is a RIC combining low-dose (2 Grays) total body irradiation, cyclophosphamide 50 mg/kg for 1 day, and fludarabine 40 mg/m^2 for 5 days (200 mg/m^2 total dose). Between June 2008 and March 2013, we performed 58 dUCB allotransplantations in our department. Among these 58 cases, only 36 patients who met the inclusion criteria for the study were identified. Twenty-two cases were not considered because they had not received the TCF regimen as conditioning or they had not been assessed for T cell chimerism within the first 100 days after transplantation. Overall, there were 19 males and 17 females. Median age for the whole cohort was 57 years (range, 17 to 69). Nineteen patients had myeloid diseases, including acute myeloid leukemia in 17 and myelodysplastic syndrome in 2 cases. Seventeen patients had lymphoid diseases, including 16 lymphomas (large diffuse B cell lymphoma [n = 5], mantle cell lymphoma [n = 2], Hodgkin disease [n = 2], B cell chronic lymphocytic leukemia [n = 2], Waldenström disease [n = 1], peripheral T cell lymphoma [n = 2], T cell prolymphocytic leukemia [n = 1], and angio-immunoblastic T cell lymphoma [n = 1]) and 1 acute lymphoblastic leukemia. Patients underwent transplantation in complete remission (n = 29) or partial remission (n = 7). Fourteen patients have been previously undergone transplantation (autologous stem cell transplantation n = 13; allogeneic stem cell transplantation n = 1). The median interval between diagnosis and graft was 10.5 months (range, 3 to 188).

All patients received cyclosporine and mycophenolate mofetil as GVHD prophylaxis. According to local practice and in case of no GVHD development, mycophenolate mofetil was tapered as early as day +30 and stopped at day +60, whereas cyclosporine was progressively tapered to be stopped between 4 to 6 months after transplantation.

Cord blood units were matched at greater than 3 of 6 HLA antigens based on antigen-level HLA-A and -B typing and allele-level HLA-DRB1 typing. Also, sufficient cryopreserved total nucleated cells (TNC) (>1.5×10^7/kg for each unit or total dose > 3×10^7/kg) was considered for the selection of CB units for all patients. The median number of TNC ($\times 10^7$/kg) and $CD34^+$ ($\times 10^6$/kg) cells infused before and after thawing were .267 (range, .135 to .492), .243 (range, .11 to .406), .071 (range, .022 to 0.427), and .042 (range, .019 to .259), respectively.

All patients gave informed consent for retrospective studies and all data were extracted from the European Society for Blood and Marrow Transplantation registry. The main objective of the study was to investigate the impact of full versus mixed TCC on survival, incidence of relapse, nonrelapse mortality, and acute or chronic GVHD after dUCB TCF allotransplantation in adults. Patients' and transplantation characteristics are summarized in Table 1.

Peripheral Blood $CD3^+$ TCC

$CD3^+$ TCC was studied on sorted peripheral blood $CD3^+$ T lymphocytes by quantitative-PCR evaluation of differential short tandem repeat DNA sequences as previously described [17]. *Full* and *mixed donor TCC* were defined by the presence of at least 95% and between 6% and 94% of $CD3^+$ donor T cells, respectively, both units combined. *Engraftment* was defined as sustained neutrophil recovery with donor chimerism > 5%, both units combined. Considering the 36 patients, 33 were evaluated for TCC at day +60 and 26 at day +100 (±10). Except for 13 cases, patients had not been evaluated for TCC at day +30 (±10) because of deep lymphopenia.

Endpoints Definition

Survival was calculated from the date of transplantation until relapse or death or last follow-up for disease-free survival (DFS) or to death or to last follow-up for overall survival (OS). Cumulative incidence of relapse (CIR) was calculated from the date of transplantation until relapse, taking into account death from any other cause as a competing risk. *Nonrelapse mortality* (NRM) was defined as death from any cause without previous relapse or progression. Acute and chronic GVHD were diagnosed and graded according to standard criteria [18,19].

Statistical Analysis

Categorical variable comparisons were performed by the Fisher exact test. Median comparisons were performed by the Mann-Whitney 2-sample

test. The impact of prognostic factors on OS and DFS was determined by univariate and multivariate Cox hazard ratio models. For CIR, the same variables were tested using the Gray test.

The Cox univariate and log rank tests were used for univariate comparisons. The following parameters were taken into account for analyses of prognostic factors for survival: age; gender; type of diseases (ie, lymphoid versus myeloid); status at transplantation; HLA matching of the engrafted cord blood unit with recipient, number of TNC, and $CD34^+$ cells infused before and after thawing (< or > median); acute GVHD grade 2 to 4 and grades 3 and 4; chronic GVHD; and full versus mixed TCC.

All factors associated with a P value < .20 by univariate analysis were included in multivariate analyses, as well as chronic GVHD for DFS ($P = .261$ in univariate analysis). Multivariate analyses were performed using the Cox model. Statistical analyses were performed using STATA software package.

RESULTS

Evaluation of TCC after Transplantation within the First 100 Days after Transplantation

All patients were evaluated at least 1 time for TCC after transplantation, whereas 24 were evaluated twice. The reasons for not being evaluated were mainly GVDH occurrence (n = 6) or technical problems with the technical assay (n = 6). If more than 1 TCC was researched, the earliest documentation of 1 full TCC was sufficient to assign patients in the complete donor TCC group. Thus, 29 cases were assigned to this former group (median donor TCC, 100%; range, 96% to 100%), whereas 7 cases were assigned to the mixed donor TCC group (median TCC, 82%; range, 14% to 94%). There were no differences between groups (Table 1). At time of TCC analyses, only 1 unit was detected in all cases and was accountable for engraftment.

Outcomes and Factors Influencing Outcomes (excluding TCC) after dUCB TCF Allotransplantation

With a median follow-up of 36 months, 3 year-OS, DFS, and CIR were 61%, (95% confidence interval [CI], 43% to 75%); 50% (95% CI, 32.5% to 66%), and 28% (95% CI, 16% to 44%), respectively. NRM was 3% at day 100 and 14% at 1 year. Incidences of grade 2 to 4 and grades 3 to 4 acute GVHD were 42% and 19.5%, respectively. Incidence of chronic GVHD was 39% (limited 28%, extensive 11%).

In univariate analysis, a younger age (studied as a continuous variable) was associated with a better DFS (hazard ration [HR], .96; 95% CI, .93 to .99; $P = .04$). No other factor was associated with better DFS, OS, or lower CIR in this study.

In multivariate analysis (Table 2), absence of chronic GVHD was associated with lower DFS (HR, 4.68; 95% CI, 1.12 to 19.53; $P = .034$). No grade 3 or 4 GVHD occurrence after transplantation and younger ager were independently associated with better OS (HR, .24; 95% CI, .06 to .93; $P = .03$; and HR, .95; 95% CI, .91 to .99; $P = .02$), respectively. On the contrary, a myeloid disease was associated with lower OS (HR, 9.13; 95% CI, 1.7 to 49.05; $P = .01$). No factor was associated with relapse incidence in the multivariate analysis.

Influence of Donor TCC within the First 100 Days after a dUCB TCF Allotransplantation in Adults

In univariate analysis, documentation of at least 1 full donor TCC within the first 100 days after transplantation was associated with better DFS (Figure 1A) (3-year DFS, complete donor TCC: 59% [95% CI, 39% to 75.5] versus mixed TCC: 14% [95% CI, 7% to 46%]; HR, .24; 95% CI, .09 to .65; $P = .005$), and lower incidence of relapse (Figure 1B) (3-year CIR, complete donor TCC: 24% [95% CI, 21.5% to 57%] versus mixed TCC: 78% [95% CI, 52% to 99%]; HR, .18; 95% CI, .05 to .5; $P = .004$). In multivariate analysis, full donor TCC remained independently associated with lower relapse incidence (HR, .17; 95% CI, .029 to .99; $P = .049$). Relapse occurred in median 51 days (range, 21 to 781) after the assessment of mixed TCC.

Table 2
Multivariate Analysis for DFS, OS, and CIR

Outcome	HR	95% CI	*P* Value
DFS			
Age (continuous variable)	.97	.93-1.01	.174
Sex	.37	.10-1.26	.111
TCC full versus mixed	.28	.074-1.04	.058
Chronic GVHD: no versus yes	4.68	1.12-19.53	.034
OS			
Age (continuous variable)	.95	.91-.99	.022
Myeloid versus lymphoid	9.13	1.7-49.05	.010
Acute GVHD: none versus grade 3-4	.24	.06-.93	.038
TCC full versus mixed	.62	.15-2.46	.495
CIR			
Age (continuous variable)	.95	.91-1.00	.057
Sex	.88	.15-5.26	.892
TCC full versus mixed	.17	.028-.99	.049
Chronic GVHD: no versus yes	8.19	.46-146.41	.153

TCC has no influence on hematopoietic recoveries (neutrophils or platelets), OS, NRM, or acute and chronic GVHD incidences in this series.

DISCUSSION

Our study showed that documentation of a mixed donor TCC within the first 100 days after transplantation is associated with a higher risk of relapse in adult patients receiving a standard dUCB TCF RIC allo-transplantation. Mixed donor TCC is probably also associated with lower DFS, but because of the small size of the population, the P value did not reach significance in our study in multivariate analysis. Although retrospective, our series has the advantage of studying a homogeneous population in terms of conditioning, number of cord blood units transplanted (double), patients (adults only), GVHD prophylaxis, type of hematological disorders (malignant only), and confirmed previous results observed after RIC transplantation using other source of stem cells [4-6]. Of note, when comparing characteristics of the engrafted unit between groups, no differences were observed, suggesting no influence of the winning unit in term of full or mixed chimerism in this study.

Two studies have previously looked at the influence of chimerism in the setting of cord blood transplantations. In the first, Berlung et al. found an association between complete donor TCC and higher incidence of acute GVHD. No association was found between TCC and other outcomes [20]. However, the population was very heterogeneous, as they included pediatric or adult patients with tumoral or benign hematological diseases, and mesenchymal stem cells were used as GVHD prophylaxis. The second study, by Elkaim et al., considered single umbilical cord blood myeloablative transplantation in 94 children and found that documentation of a full donor chimerism, performed on whole peripheral blood cells, was predictive of higher incidence of GVHD but with no influence on relapse [21]. Thus, our series is the first to document the influence of TCC in the setting of dUCB TCF allotransplantation, which is currently a standard of care for adults lacking a suitable donor and eligible for allotransplantation.

A diagnosis of myeloid disease was associated with lower OS in our study. One can hypothesize that in case of lymphoid diseases, where prognosis is better, normal

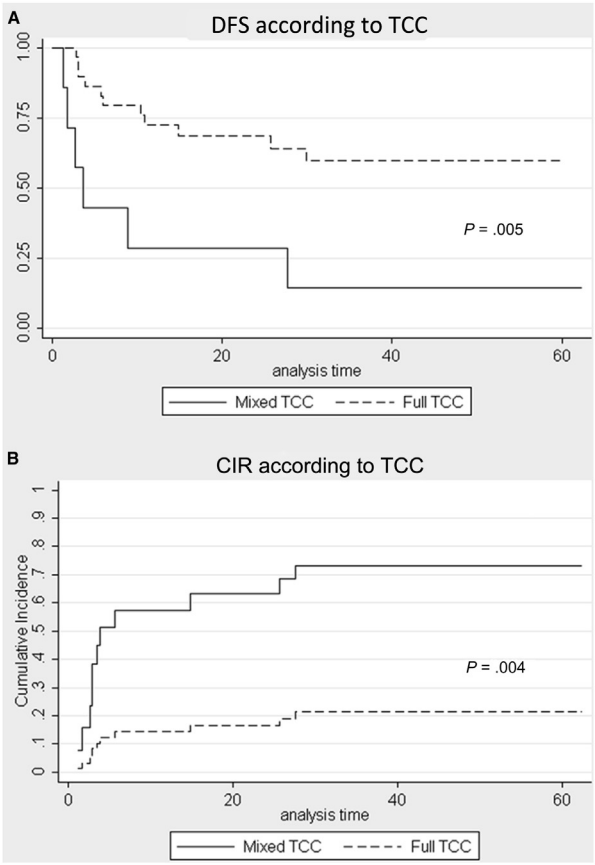

Figure 1. (A) At 3 years, DFS was estimated at 59% (95% CI, 39% to 75.5%) in the full donor TCC group versus 14% (95% CI, 7% to 46%) in the mixed TCC group; HR, .24; 95% CI, .09 to .65; $P = .005$. (B) At 3 years, CIR was estimated at 24% (95% CI, 21.5% to 57%) in the full donor TCC group versus 78% (95% CI, 52% to 99%) in the mixed TCC group; HR, .18; 95% CI, .05 to .5; $P = .004$.

tapering of immunosuppressive prophylaxis should be required to avoid too much GVHD, whereas in case of more aggressive disease, such as acute myeloid leukemia or acute lymphoblastic leukemia, with a mixed TCC within the first 100 days after transplantation, withdrawal of the drugs should be conducted more rapidly. The question of immunotherapy allowing for early conversion to full donor chimerism after umbilical cord blood allotransplantation should be also explored in the future. For example, Tomizawa et al. expanded $CD4^+$ T lymphocytes from a residue of the cord blood unit used for transplantation. Donor cord blood lymphocytes infusion in patients improved conversion to a full donor chimerism after transplantation [22].

Finally, advanced aged was associated with worse overall survival in our series. It confirms the worse prognosis of older patients after umbilical cord blood allotransplantation, as reported by Labopin et al. recently [23].

In conclusion, in our study, documentation of a complete donor $CD3^+$ TCC is independently associated with a lower risk of relapse in adults receiving a standard dUCB TCF RIC allotransplantation. These results should be confirmed by other studies, and immunotherapy, allowing for early conversion to full donor chimerism after this type of graft, should be developed in the future.

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Patients with Philadelphia-Positive Leukemia with BCR-ABL Kinase Mutations before Allogeneic Transplantation Predominantly Relapse with the Same Mutation

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Despite the successes of tyrosine kinase inhibitors (TKIs) in improving outcomes in patients with chronic myeloid leukemia (CML) and Philadelphia-positive acute lymphoblastic leukemia (Ph + ALL), allogeneic hematopoietic stem cell transplantation (HSCT) continues to be an important and potentially curative option for selected patients with either disease. After HSCT, TKIs are increasingly being used to treat or prevent disease relapse, and practice patterns suggest that these TKIs are often chosen empirically without regard to pre-HSCT mutation status. We investigated whether ABL kinase domain mutations persist after transplantation and, thus, whether pre-HSCT mutation status should inform the selection of post-HSCT TKIs in these patients. We retrospectively analyzed adults who underwent allogeneic HSCT for CML and Ph + ALL at our institution between 2000 and 2010, and we identified subjects who had detectable BCR-ABL transcripts by polymerase chain reaction (PCR), as well as available RNA for Sanger sequencing of the ABL kinase domain, in both the pre- and post-HSCT settings. In total, 95 CML and 20 Ph + ALL patients with positive PCR transcripts were identified, of which 10 (10.5%) and 4 (20.0%), respectively, were found to have pre-HSCT ABL kinase mutations known to confer TKI resistance. In 9 (64.2%) of these 14 patients, the same kinase mutation was also detectable at an average time of 191 days after HSCT. Seven (50.0%) of the 14 harboring mutations had relapsed/refractory disease by last follow-up, of which, in retrospect, 6 had received a predictably ineffective TKI within the first 100 days after transplantation based on our mutation analysis. These data support the idea that pre-existing mutations in the ABL kinase domain, frequently associated with resistance to TKIs and prevalent in a transplantation population, are persistently detectable in the majority of patients after transplantation. We propose that such resistance patterns should be considered when selecting TKIs in the post-HSCT setting, including clinical trials of post-HSCT TKI prophylaxis.

INTRODUCTION

Tyrosine kinase inhibitors (TKIs) remain the front-line therapy for patients with chronic myeloid leukemia (CML) and also improve outcomes when incorporated into induction and maintenance regimens for those with Philadelphia-positive acute lymphoblastic leukemia (Ph + ALL). Resistance to TKIs is commonly due to the emergence of clones containing point mutations in the ABL kinase domain of BCR-ABL, occurring in as many as 30% to 60% of CML patients with imatinib resistance [1,2], as well as in over one third of Ph + ALL patients at the time of diagnosis [3]. More than 100 ABL kinase domain mutations associated with TKI resistance have been described [4].

In the current treatment approach for CML, allogeneic hematopoietic stem cell transplantation (HSCT) is generally reserved for patients who fail or are intolerant of TKI therapy or for those with advanced phase disease. Transplantation is also a potentially curative option for Ph + ALL patients with HLA identically matched related or unrelated donors. Relapse after HSCT is fairly common in both advanced phase CML (30% to 40%) [5,6] and Ph + ALL (30% to 60%) [7]. Therapeutic strategies to treat post-HSCT relapse historically consisted of withdrawal of immunosuppression or donor lymphocyte infusions, though several studies have shown efficacy in treating relapsed CML or Ph + ALL with TKIs [8-15]. An increasing number of studies have also evaluated the prophylactic use of TKIs after transplantation in high-risk patients, including those with detectable BCR-ABL at the time of transplantation, though there are very limited data to guide their selection and administration [16-19].

In addition to cost considerations, the selection of post-transplantation TKIs is based, at least in part, on toxicity profiles of the available agents. The bulk of retrospective and prospective studies using imatinib indicate that it is generally well-tolerated after HSCT, even in the early

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